# Nephrocalcinosis and Blood Chemistry in Mature Rainbow Trout (*Salmo gairdneri*)

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# NEPHROCALCINOSIS AND BLOOD CHEMISTRY IN MATURE RAINBOW TROUT (*Salmo gairdneri*)

by

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## ABSTRACT

Yurkowski, M., D.C. Gillespie, D.A. Metner, and W.L. Lockhart. 1985. Nephrocalcinosis and blood chemistry in mature rainbow trout (Salmo gairdneri). Can. Tech. Rep. Fish. Aquat. Sci.: iv + 7 p.

The severity of nephrocalcinosis in rainbow trout (Salmo gairdneri) was categorized into six stages of severity based on arbitrary visual characteristics.

The blood and blood serum parameters were determined in sexually mature unspawned male and female rainbow trout. With increasing severity of nephrocalcinosis, the blood hematocrit values and hemoglobin content decreased as did serum sterol and lipid concentrations, while the glutamic oxalacetic transaminase (GOT) activities, creatinine concentrations, and moisture, all increased significantly ($P<0.05$). The levels of most serum n-6 and n-3 fatty acids increased but that of 18:0, 16:1, 20:1, and 22:1 decreased significantly. Results suggest that in severe nephrocalcinosis most of the functional kidney tissue is destroyed resulting in some impairment of kidney function.


Key words: nephrocalcinosis; serum; Salmo gairdneri; creatinine; lipids; fatty acids; enzymes; haemoglobins; proteins; minerals.


## RESUME

Yurkowski, M., D.C. Gillespie, D.A. Metner, and W.L. Lockhart. 1985. Nephrocalcinosis and blood chemistry in mature rainbow trout (Salmo gairdneri). Can. Tech. Rep. Fish. Aquat. Sci.: iv + 7 p.

On a classifié la virulence de la néphrocalcinose de truites arc-en-ciel (Salmo gairdneri) en six degrés de virulence jugée selon des caractéristiques visuelles arbitraires.

Les paramètres du sang et du sérum sanguin furent déterminés dans des truites arc-en-ciel mâles et femelles ayant atteint leur maturité sexuelle, mais n'ayant pas frayé. Au fur et à mesure que la néphrocalcinose s'aggravait, les valeurs de l'hématocrite sanguin et du contenu en hémoglobine diminuait, ainsi que les concentrations de lipides et de stérol du sérum, et les activités de la glutamique-oxaloacétique-transaminase (GOT), les concentrations de créatine et l'humidité augmentaient sensiblement ($P<0,05$). Le niveau d'acides gras n-6 et n-3 de la plupart des sérums augmentait, mais celui des 18:0, 16:1, 20:1, et 22:1 diminuait sensiblement. Les résultats semblent indiquer que dans les cas de néphrocalcinose virulente, une grande partie des tissus rénaux fonctionnels sont détruits par suite d'un mauvais fonctionnement du rein.


Mots-clés: néphrocalcinose; sérum; Salmo gairdneri; créatine; lipides; acides gras; enzymes; hémoglobine; protéine; minéraux.

## INTRODUCTION

A number of incidents of nephrocalcinosis in rainbow trout (Salmo gairdneri) have been reported from Europe and North America (Wunder 1967; Besse et al. 1968; Smith et al. 1974; Landolt 1975; Cowey et al. 1977; Schlotfeldt 1980). A particularly severe outbreak of nephrocalcinosis was reported in sexually mature rainbow trout at the Experimental Hatchery, Rockwood, Manitoba (Gillespie and Evans 1979; Schlotfeldt 1980). In severe cases of nephrocalcinosis, we and Landolt (1975) have observed that most of the functional kidney tissues appeared to be destroyed. Therefore in this paper the different stages of nephrocalcinosis occurring in these fish are described. In addition, the influence of differing severities of nephrocalcinosis on blood chemistry of sexually mature male and female rainbow trout is described to indicate any loss in kidney function and any other clinical changes.

## MATERIALS AND METHODS

Brood rainbow trout, Pennask strain (originating from Pennask Lake, British Columbia) were raised at the Rockwood Experimental Hatchery from eyed eggs from July 22, 1971, to January 20, 1976. Fish were fed commercial feed from unknown sources. From this mature unspawned fish stock, with different stages of development nephrocalcinosis, samples were taken on January 20, 1976, which was past the normal spawning period. Blood samples were obtained from caudal vessels of fish under anaesthesia (phenoxyethanol-2) by the method of Steucke and Schoettger (1967) and allowed to clot on ice. The fish were then examined to visually determine sex, gonadal maturity (condition) and the severity of nephrocalcinosis. The serum samples were selected for analysis based upon the disease classificiation, viz., normal, borderline stage and stage 1 to stage 5 (see description under RESULTS AND DISCUSSION). Stages available in sufficient numbers for analyses, from both sexes in equal numbers, were borderline, stage 1 and stage 4. Serum inorganic phosphorus, total phosphorus, protein, glucose, creatinine, urea, nitrogen, sodium, potassium, calcium and chloride, and the activity of LDH (lactate dehydrogenase, EC. 1.1.1.27), GOT (glutamic oxalacetic transaminase, EC.1.6.1.1) and ACHE (cholinesterase, acetylcholine acyl hydrolase, EC.3.1.1.8) were determined as previously described (Lockhart et al. 1975). Hematocrit and hemoglobin (cyanmethemoglobin method) concentrations in whole blood were also determined. Serum moisture content was determined by desiccation under calcium chloride to a constant weight. The serum total lipid content was determined by the vanillin colorimetric method (Boehringer Mannheim Kit, Cat. No. 15991), and results were confirmed by the gas-liquid chromatographic (GLC) method described below.

The serum was analyzed for total lipid, total sterol, total fatty acid and total phospholipid content, and fatty acid composition as described below. Total lipids were extracted from the serum by the method of Bligh and Dyer (1959), after an internal standard (2.50 mg n-tetratriacontane, Analabs Inc., in 1 mL of chloroform) was added to 1 mL of serum. Lipid extracts were analyzed for phosphorus (Parker and Peterson 1965). The phospholipid content was calculated by multiplying the phosphorus value by 25. Lipids were transesterified and a portion, containing the internal standard, was used to determine the total sterols and total lipid content as described by Yurkowski et al. (1978). In the present work, the GLC column temperature was 235°C. The transesterified lipids were purified and their fatty acid composition was similarly determined (Yurkowski et al. 1978), except the column packing was GP 10% SP-2300 on 80/100 mesh Supelcoport and the column temperature was 160°C. The individual fatty acids were identified by use of standard fatty acids, by plots of log relative retention times versus chain-length and by comparison of equivalent chain lengths as described by Jamieson (1975).

Results between male and female trout and between borderline and stage 1 nephrocalcinosis were not statistically different as indicated by the Student's t-test comparison (Snedecor and Cochran 1980). Therefore the data obtained were placed in two nephrocalcinotic groups (each containing equal number of males and females), one group with minor signs (borderline and stage 1) and a second group with major signs (stage 4). Mean values then were compared using Student's t-test.

## RESULTS AND DISCUSSION

### DESCRIPTION OF STAGES OF NEPHROCALCINOSIS

The photographs of kidney tissues of mature rainbow trout showed that nephrocalcinosis occurred in various stages of development, which have been arbitrarily classified as normal kidney (Fig. 1A), borderline stage (Fig. 1B) and stages 1-5 (Fig. 1C-1G) by visual examinations. The visual description of different stages is presented in Table 1. This disease appears to be non-infective (Landolt 1975) and begins at the borderline stage with a trace deposit of calcium in the posterior mesonephric ducts of the kidney; this was shown by x-ray examinations (Max Blouw, University of St. Francis Xavier, Antigonish, Nova Scotia, personal communication). Previously, Gillespie and Evans (1979) observed that our stock of fish had lesions of nephrocalcinosis, similar to those reported by Wunder (1967). Their histological sections and isolated granules from the kidney tubules show that mineral deposits (containing calcium, magnesium, phosphorus and carbonate) form a network embedded in a ceroid matrix. These lesions progressively increased in size until most of the functional kidney tissue was destroyed (see Fig. 1F-1G; Landolt 1975). Since this classification is preliminary and arbitrary, further studies on the classification of nephrocalcinosis by histological examination under controlled nutritional and environmental conditions should be done.

INFLUENCE OF NEPHROCALCINOSIS ON BLOOD AND BLOOD SERUM PARAMETERS

Blood chemical measurements from two groups of sexually mature rainbow trout (males and females equally represented) with slight signs and with severe signs of nephrocalcinosis, sampled January 20, were compared (Table 2).

Hematocrit and hemoglobin

Increasing severity of nephrocalcinosis was associated with a significant decrease in the hematocrit values and hemoglobin content in the blood (Table 2). An explanation for the large decrease in hemoglobin is uncertain. Previous studies have implicated pyridoxine (Smith et al. 1974) and phosphorus (Andrews et al. 1973) deficiencies, fasting (Kawatsu 1966) and infection (Shieh and MacLean 1976). Increase in nephrocalcinosis severity produced degeneration of the hematopoietic tissues located in the anterior portion of the kidney (Anderson et al. 1976; see also Fig. 1A-1G), but no effect on the spleen was reported. It could be reasonably postulated that this degeneration slowed the replacement of hemoglobin.

Moisture and protein

Serum moisture was consistently higher in fish with severe signs (95.6%) than in fish with no overt signs (93.9%) ($P \leq 0.05$) of nephrocalcinosis. There appeared a corresponding drop in proteins, which was not statistically significant. Presumably this reflects impaired excretion of water by the kidneys, but this serum with excess water was not depleted in major ions.

Major ions

Nephrocalcinosis had no significant effect on serum chloride, inorganic phosphorus, calcium, magnesium and potassium. Regulation of major serum ions was apparently unaffected by the severity of nephrocalcinosis, thus, some aspects of kidney function were retained.

Urea and creatinine

The blood urea nitrogen (BUN) content in the blood serum was not influenced by nephrocalcinosis of rainbow trout (Table 2). Infection in rainbow trout (columnaris disease) (Lockhart and Metner 1984), and brook trout (furunculosis) (Shieh and MacLean 1976), and urinary obstruction, nephritis and chronic glomerulonephritis in man (Searcy 1969), increase this parameter. In fish both gills and kidneys can excrete urea (Delaunay 1929; Smith 1929) and it appears that with nephrocalcinosis, the gills probably remain functional and can aid the diseased kidney in the clearance of urea.

The creatinine concentration in the serum increased by about four fold in fish with severe nephrocalcinosis (Table 2). In man, elevated creatinine concentrations in the blood indicate renal dysfunction resulting from failure to clear creatinine into the urine (Searcy 1969). In fish, the kidney, but not the gills, excrete creatinine (Delaunay 1929; Smith 1929). This elevation in serum creatinine argues that the gross kidney deformations, shown in Fig. 1A-1G, are accompanied by impairment in the excretion function. Landolt (1975) described nephrocalcinosis in rainbow trout as renal deposits accompanied by tubular dilations and degeneration, the formation of tubular casts and, in extreme cases, the loss of almost all kidney function.

Enzymes

The severity of nephrocalcinosis had no effect on LDH and ACHE activities, but GOT activity approximately doubled (Table 2). This increase is statistically meaningful, but we are uncertain of its biological interpretation. Increased GOT activity has been reported in fish suffering from a bacterial disease (Bell 1968) and chemical poisoning (Lockhart et al. 1975; Lockhart and Metner 1984). In man, increased GOT activity can indicate renal infarction and other degenerative diseases (Searcy 1969; Kachmar 1970). Rainbow trout with nephrocalcinosis probably were not in a fasting state because they were given feed and GOT activity decreased in fasting trout (Sauer and Haider 1979).

Lipids

The lipid and sterol content of sera, but not phospholipid content, of the rainbow trout decreased significantly with the increase in severity of nephrocalcinosis (Table 2). Indirectly, this showed the lipid content decrease was primarily due to triglycerides. During fasting, increases occur in the content of cholesterol and free fatty acids in rainbow trout sera (Shibata et al. 1974) and cholesterol and lipoproteins in human sera (Searcy 1969). During furunculosis infection, the content of triglycerides, fatty acids and lipid-phosphorus decreases in brook trout (Shieh and MacLean 1976). This suggests that fasting and infection may not be involved in nephrocalcinosis. In addition, our results suggested that in stage 4 nerphrocalcinosis, renal function is not completely shut down. As an example of many studies, Bagdade et al. (1978) report that in complete renal failure in rats a large lipid increase occurs in the serum, but in rainbow trout with severe nephrocalcinosis (stage 4) a decrease was observed.

Fatty acid composition

The levels (weight %) of saturated (18:0) and monoenoic (16:1; 20:1; 22:1) fatty acids decreased significantly, while all polyenoic fatty acids, except 22:6n-3, increased significantly in rainbow trout with increase in disease severity (Table 2). The levels of 16:0, 18:1 and 22:6n-3 remained constant. In general, the total of saturated plus monoenoic fatty acids decreased, while the total n-6 and total n-3 fatty acids increased with the severity of the disease. The higher levels of serum polyenoic fatty acids in more severe nephrocalcinosis can

be attributed to lower levels of triglycerides with little change in the levels of phospholipids, which are more unsaturated. The lower triglyceride levels and decreased concentration (weight/unit protein) of 16:0, 16:1, 20:4n-6, 22:6n-3 and other minor polyenoic (20:3n-6; 18:4n-3; 22:5n-3) fatty acids (data on weight/unit protein basis were not presented),suggested that the severity of nephrocalcinosis had some influence on the mobilization and use of serum lipids, which may be reflected in the content and composition of serum lipoproteins and their lipids, including fatty acids of lipids. However, the reason for lower cholesterol and triglyceride content in serum of rainbow trout with severe nephrocalcinosis and observed gonad resorption is not clear.

## ACKNOWLEDGMENTS

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## REFERENCES

ANDERSON, C.D., R.J. ROBERTS, K. MacKENZIE, and A.H. McVICAR. 1976. The hepatorenal syndrome in cultured turbot (Scophthalmus maximum L.). J. Fish Biol. 8: 331-341.

ANDREWS, J.W., T. MURAI and C. CAMPBELL. 1973. Effects of dietary calcium and phosphorus on growth, food conversion, bone ash and hematocrit levels of catfish. J. Nutr. 103: 766-791.

BAGDADE, J.D., E. YEE, D.E. WILSON, and E. SHAFRIR. 1978. Hyperlipidemia in renal failure: studies of plasma lipoproteins, hepatic triglyceride production, and tissue lipoprotein lipase in a chronically uremic rat model. J. Lab. Clin. Med. 91: 176-186.

BELL, G.R. 1968. Distribution of transaminases (aminotransferases) in the tissues of Pacific salmon (Oncorhynchus) with emphasis on the properties and diagnostic use of glutamic oxaloacetic transaminase. J. Fish. Res. Board Can. 25: 1247-1268.

BESSE, P., P. de KINKELIN, and J.C. LEVADITI. 1968. La néphrocalcinose dans la truite arc-en-ciel (Salmo irideus Gibbons 1855). Bull. Off. Int. Epizoot. 69: 1391-1399.

BLIGH, E.G., and W.J. DYER. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37: 911-917.

COWEY, C.B., D. KNOX, J.W. ADRON, S. GEORGE, and B. PIRIE. 1977. The production of renal calcinosis by magnesium deficiency in rainbow trout (Salmo gairdneri). Br. J. Nutr. 38: 127-135.

DELAUNAY, H. 1929. Sur l'excrétion l'azotée des poissons. C.R. Séances Soc. Biol. Fil. 101: 371-372.

GILLESPIE, D.C., and R.E. EVANS. 1979. Composition of granules from kidneys of rainbow trout (Salmo gairdneri) with nephrocalcinosis. J. Fish. Res. Board Can. 36: 683-685.

JAMIESON, G.R. 1975. GLC identification techniques for long-chain unsaturated fatty acids. J. Chromatogr. Sci. 13: 491-497.

KACHMAR, J.F. 1970. Enzymes, p. 362-473. In N.W. Tietz (ed.) Fundamentals of clinical chemistry. Saunders, Philadelphia, PA.

KAWATSU, H. 1966. Studies on the anemia of fish. 1. Anemia of rainbow trout caused by starvation. Bull. Freshwater Res. Lab. Tokyo 15: 167-173.

LANDOLT, M.L. 1975. Visceral granuloma and nephrocalcinosis of trout. p. 793-800. In W.A. Ribelin and G. Migaki (ed.). The pathology of fishes. University of Wisconsin Press, Madison, WI.

LOCKHART, W.L., and D.A. METNER. 1984. Fish serum chemistry as a pathology tool, p. 73-85. In V.W. Cairns et al. (ed.) Contaminant effects on fisheries. Wiley, New York, NY.

LOCKHART, W.L., R. WAGEMANN, J.W. CLAYTON, B. GRAHAM, and D. MURRAY. 1975. Chronic toxicity of a synthetic tri-aryl phosphate oil to fish. Environ. Physiol. Biochem. 5: 361-369.

PARKER, F., and N.F. PETERSON. 1965. Quantitative analysis of phospholipids and phospholipid fatty acids from silica gel thin-layer chromatography. J. Lipid Res. 6: 455-460.

SAUER, D.M., and G. HAIDER. 1979. Enzyme activities in the plasma of rainbow trout, Salmo gairdneri Richardson; the effects of nutritional status and salinity. J. Fish Biol. 14: 407-412.

SCHLOTFELDT, H.-J. 1980. The increase of nephrocalcinosis in rainbow trout in intensive aquaculture, p. 185-205. In W. Ahne (ed.) Fish diseases. Springer Verlag, New York, NY.

SEARCY, R.L. 1969. Diagnostic biochemistry. McGraw-Hill, New York, NY. 660 p.

SHIBATA, N., T. KINUMAKI, and H. ICHIMURA. 1974. Triglyceride, cholesterol, free fatty acid, glucose and protein contents in plasma of cultured rainbow trout. Bull. Tokai Reg. Fish. Res. Lab. 77: 77-87.

SHIEH, H.S., and J.R. MacLEAN. 1976. Blood changes in brook trout induced by infection with Aeromonas salmonicida. J. Wildl. Dis. 12: 77-82.

SMITH, H.W. 1929. The excretion of ammonia and urea by the gills of fish. J. Biol. Chem. 81: 727-742.

SMITH, C.E., M. BRIN, and J.E. HALVER. 1974. Biochemical, physiological and pathological changes in pyridoxine-deficient rainbow trout (Salmo gairdneri). J. Fish. Res. Board Can. 31: 1893-1898.

SNEDECOR, G.W. and W.G. COCHRAN. 1980. Statistical methods, 7th ed. Iowa State University Press, Ames, IA. 507 p.

STEUCKE, E.W. Jr, and R.A. SCHOETTGER. 1967. Comparison of three methods of sampling trout blood for measurements of hematocrit. Prog. Fish-Cult. 29: 98-101.

WUNDER, W. 1967. Eigenartige Veranderungen an der Niere und die Ausbildung einer Nierenzyste bei der Regenbogenforell. (Peculiar renal changes and the formation of a renal cyst in the rainbow trout.) Allg. Fisch-Ztg. 95(15): 470-471. (Can. Fish. Mar. Serv. Transl. Ser. No. 3196, 1974)

YURKOWSKI, M., J.K. BAILEY, R.E. EVANS, J.L. TABACHEK, G.B. AYLES, and J.G. EALES. 1978. Acceptability of rapeseed proteins in diets of rainbow trout (Salmo gairdneri). J. Fish. Res. Board Can. 35: 951-962.

Table 1. Description of different stages of nephrocalcinois in mature rainbow trout.

| Stages of nephrocalcinois | Description |
|---|---|
| normal[a] | - two parallel mesonephric ducts; barely visible with no white coloration (Fig. 1A) |
| borderline[a] | - two mesonephoric ducts; visible with white coloration; no dilations (Fig. 1B) |
| 1 | - two mesonephric ducts; white coloration; dilated (Fig. 1C) |
| 2 | - two mesonephric ducts; white coloration; dilated and convoluted (Fig. 1D) |
| 3 | - kidney appeared necrotic and calified; no enlargement (Fig. 1E) |
| 4 | - kidney appeared necrotic and calcified; enlarged to fill one-half of body cavity (Fig. 1F) |
| 5 | - kidney appeared necrotic and calcified; enlarged to fill almost the entire body cavity (Fig. 1G) |

[a] To differentiate normal kidney from borderline nephrocalcinosis, the mesonephric ducts must be examined before the fish are bled, that is, with an intact blood recirculation system.

Table 2. Effect of severity of nephrocalcinosis on the biochemical parameters of blood sera from mature rainbow trout.

| Parameters[a] | Severity of visual signs of nephrocalcinosis | | Significance[b] |
|---|---|---|---|
| | Slight (borderline & stage 1) | Extreme (stage 4) | |
| Hematocrit, % | 34.15±3.63(13)[c] | 21.86±5.55(7) | ** |
| Hemoglobin, $mg \cdot L^{-0.1}$ | 8.28±1.14(13) | 5.76±1.50(7) | ** |
| Moisture, $mg \cdot L^{-0.1}$[d] | 93.9 ±1.14(11) | 95.6 ±1.94(6) | * |
| Creatinine, $mg \cdot L^{-0.1}$ | 0.20±0.07(12) | 0.77±0.21(7) | ** |
| GOT, mU/mg protein | 4.95±1.94(13) | 9.13±4.84(7) | * |
| Total lipid, $mg \cdot L^{-0.1}$ | 1333±505(13) | 696±316(7) | * |
| Total sterols, $mg \cdot L^{-0.1}$ | 259± 83(12) | 162± 39(6) | * |
| Fatty acids[d], wt % | | | |
| 16:0 | 18.79±1.19(6) | 17.43±0.82(6) | NS |
| 18:0 | 3.09±0.61(12) | 2.73±0.71(6) | ** |
| 16:1 | 5.33±0.72(12) | 4.35±0.48(6) | ** |
| 18:1 | 17.46±2.42(12) | 15.27±1.48(6) | NS |
| 20:1 | 3.49±0.86(12) | 3.05±1.10(6) | ** |
| 22:1 | 1.88±0.75(12) | 1.40±0.89(6) | ** |
| 18:2n-6 | 6.63±1.16(12) | 8.06±1.47(6) | ** |
| 18:3n-6 | 0.61±0.17(12) | 1.03±0.54(6) | ** |
| 20:3n-6 | 0.56±6.13(12) | 0.39±0.12(6) | ** |
| 20:4n-6 | 3.87±1.18(12) | 4.99±0.76(6) | ** |
| 18:3n-3 | 0.24±0.06(12) | 0.27±0.10(6) | ** |
| 18:4n-3 | 0.25±0.09(12) | 0.36±0.17(6) | ** |
| 20:3n-3 | 1.60±0.36(12) | 1.70±0.28(6) | ** |
| 20:5n-3 | 6.14±1.54(12) | 8.07±1.56(6) | ** |
| 22:5n-3 | 1.40±0.49(12) | 1.87±0.71(6) | ** |
| 22:6n-3 | 25.08±3.73(12) | 25.2 ±2.67(6) | NS |
| Saturated + monoenoic acids[e] | 52.07±4.23(12) | 45.90±3.2 (6) | * |
| n-6 acids | 11.66±1.39(12) | 12.89±1.81(6) | ** |
| n-3 acids | 34.70+4.83(12) | 39.50±4.59(6) | * |

[a] Other parameters measured which were not significantly different ($P>0.05$), excluding fatty acids (16:0; 18:1, 22:6n-3) are listed here. They are protein, glucose, BUN, chloride, total phosphorus, inorganic phosphorus, calcium, magnesium, potassium, ACHE, LDH, phospholipids and 14:0. Minor fatty acids present (<0.5%) are 12:0, 13:0, 15:0, 15:0 anteiso, 20:0, 23:0, 14:1, 15:1, 17:1, 24:1, 16:2, 20:2n-6 (20:3n-6), 22:2n-6, 22:4n-6; 22:5n-6, 22:3n-3, 20:4n-3 and 22:4n-3.

[b] NS denotes $P>0.05$, *denotes $P\leq 0.05$ and ** denotes $P\leq 0.01$.

[c] Mean ± standard deviation (number of fish).

[d] Fatty acids are presented in short-hand. For example, linoleic acid was denoted by 18:2n-6, which indicates a molecule with 18 carbon atoms with two methylene-interrupted bonds of which one occurs 6 carbons from the terminal end.

[e] The levels of total saturated fatty acids and total monoenoic fatty acids each tended to decrease with increase in nephrocalcinosis severity, but the difference was not statistically significant.

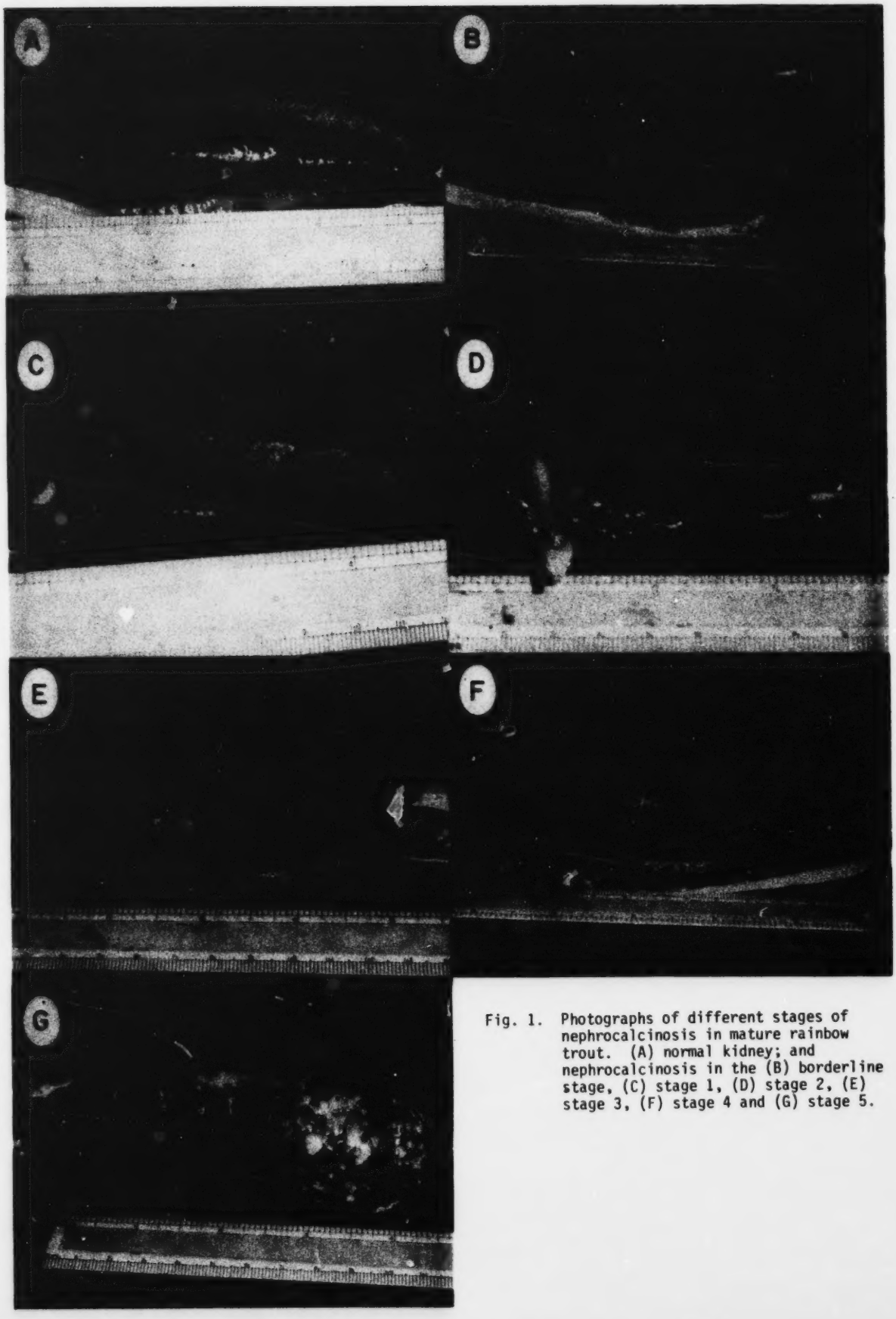


Fig. 1. Photographs of different stages of nephrocalcinosis in mature rainbow trout. (A) normal kidney; and nephrocalcinosis in the (B) borderline stage, (C) stage 1, (D) stage 2, (E) stage 3, (F) stage 4 and (G) stage 5.